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Research Article

Cortisol, Testosterone and Soccer: Hormonal Trends through a Competitive Season

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Abstract

Activation of the hypothalamic–pituitary–adrenal axis usually occurs after physical and/or psychological stress with a magnitude modulated by both intensity of the stress episodes and individual variability. Competitive sport activities can be considered as a stressor, and many studies have investigated the dynamic relationships of the hypothalamic–pituitary–adrenal axis finding different results. The aim of our study was to evaluate cortisol and testosterone levels in salivary samples from 16 soccer players over six training sessions and 18 matches. Resting levels of cortisol show pre-match levels greater than pre-training levels, with both increasing through the season. Cortisol changes following training and match sessions show lower variations for training sessions than matches. Through the season, training sessions show reduced increases in cortisol levels. For testosterone, pre-match and pre-training levels decrease through the season. Testosterone responses to training sessions and matches are different: training increases and matches decrease the testosterone levels. Through the season, matches result in reduced testosterone decreases.

In conclusion, our data show that changes induced by training sessions and matches on the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–testis axes are significantly different, and show different trends through the season. Therefore, evaluation of adrenal and testis function in soccer players should take into account differences between the intensity of effort in training sessions and matches, as well as the period of the season under study.

Keywords: Adrenal, Pituitary, Stress, Competition, Training

Introduction

Activation of the hypothalamic–pituitary–adrenal axis usually occurs after physical and/or psychological stress [1]. The magnitude of this activation is modulated by both intensity of the stress episodes and individual variability. Competitive sport activities can be considered as a stressor [2], and many studies have investigated the dynamic relationships of the hypothalamic–pituitary–adrenal axis in both individual and team sports. Amongst the team sports, soccer has been widely investigated both in males and females, at different levels, and in different contexts. At the same time, the hypothalamic–pituitary–testis axis can also be modulated by physical exercise and by aggressiveness, which are both typical of competitive sports, and of soccer in particular [3]. Cortisol, testosterone and dehydroepiandrosterone sulfate (DHEA-S) have therefore been studied as representative indices for adrenal and testis function [1]. The importance of monitoring cortisol, testosterone and DHEA-S in athletes is related with both their physiological effects and powerful of physical exercise to modulate their production, according to type and volume of training. Indeed, when psycho-physical stress is too high, a catabolic state, characterized by chronic high cortisol levels, predominates the anabolic state, supported by both testosterone and DHEA-S production, determining the worsening of both health and performance because of, for example, increased proteolysis and muscle wasting and both lipolysis and immune response suppression [4].

As soccer represents both psychological and physical stress, lasting approximately 10 months, for the hypothalamic–pituitary–testis axis, and as psychological and physical stress have a different modulation during the different phases of the season (i.e. pre-competitive season, competitive season), it is interesting to know their effect on endocrine system in order to avoid a catabolic state and to optimize the performance.

Soccer is classified as an intermittent sport, as it shows alternating aerobic/anaerobic stress. During soccer matches, the players are subjected to efforts of variable intensities [5] high-intensity and lower-intensity efforts are interspersed with active recovery [6]. Indeed, depending on their playing position players keep still for 7 min to 13 min (of a 90-minute match), while the remaining time is spent running at variable speeds (Table 1) [7]. The mean distance that a player will run during a soccer match has been reported as 13.16 ± 2.45 km [8,9].

Player position	Still (min:sec)	Running (%total running time)				Total distance covered (m)
		<10 km/h	10-13 km/h	13-18 km/h	>18 km/h	
Defender	13:30 ±3:36	51	15	18	15	10311 ±361
Midfielder	7:11 ±2:55	47	19	25	15	11082 ±653
Forward	13:16 ±7:20	45	16	23	15	9421 ±549

Table 1. Characteristics of the physical effort according to run intensity for soccer players over a 90-minute match (from Bellotti & Matteucci, 1999).

Combination of these different phases of exercise will therefore result in effort and stress of variable intensity and magnitude. This particularity of soccer appears to have led to studies that show different results with respect to the cortisol and testosterone levels of the players. Thus several studies have shown changes in cortisol and testosterone levels after a soccer match [10-12] while others have failed to show any significant change [13,14].

Most of these studies, however, were not homogenous, as they considered single matches [10,14] competitive training matches [13], or single training sessions [11]. Similarly, some studies evaluated top-level (i.e. professional) players [13], while others were based on collegiate or amateur players [10,11]. Also, few of these studies considered the changes in testosterone and cortisol levels through a soccer season, with most based on a few samples taken during intensive training [15] or during the match season. Therefore, to our knowledge, there have been no studies that have evaluated repeated measures of testosterone and cortisol levels of salivary samples before and after both training sessions and matches through a whole competitive season.

In order to reconcile the different results available in relation to cortisol, testosterone and DHEA-S levels, the aim of our study was to evaluate adrenal and testis response to soccer trainings and matches throughout a competitive season aiming to test the hypothesis that the timing (within the season) of the sampling might significantly affect the obtained results. Finally, comparative analysis of the data obtained from trainings and matches in close temporal sequence was performed to evaluate physiological response to a different stimulus.

Materials and Methods

Study population

Sixteen soccer players of the Pro Vasto Football Club, aged from 18 to 35 years, participated in this study on a voluntary basis. The study was approved by the Ethical Committee of the "G. D'Annunzio" University of Chieti–Pescara, Italy. Written informed consent of each participant was obtained. The saliva samples were obtained through the competitive season, which consisted of 34 matches and lasted from September to May. The statistical analyses were performed only where ≥ 10 players had provided saliva samples both before and after the training sessions or matches and at the same time took part in matches for at least 45 min. Therefore, six training sessions and 12 matches were evaluated, as listed in Table 2.

Hormone assays

The saliva samples were collected 30 minutes before and 30 minutes after the training sessions, and 30 minutes before the pre-match warm-up periods and 30 minutes after the matches. All of the training sessions started at 15:00 hours and all of the matches started at 14:30 hours, according to the regulations of the Italian Soccer Federation. All matches were played on Sunday and lasted 90 minutes plus injury time; all evaluated trainings occurred on Thursday and lasted 90 ± 10 minutes. The players were told to avoid drinking and eating for at least 1 h before collection of the samples, while they were encouraged to drink during the intervals of both matches and training that occurred at halftime. The saliva samples were collected in Salivette devices (Sarstedt, Germany), using sterile cotton swabs that were placed in the mouth and chewed for 1 min; the saliva samples were then squeezed into conical tubes.

dd/mm	Training session	Match	Pair
02/11	-	1 - home	--
27/11	I [a]	-	
30/11	-	2 [a] - home	A
11/18	II [b]	-	
14/18	-	3 [b] - home	B
26/02	III [c]	-	
01/03	-	4 [c] - away	C
08/03	-	5 - home	--
22/03	-	6 - home	--
29/03	-	7 - away	--
02/04	IV [d]	-	
05/04	-	8 [d] - home	D
19/04	-	9 - away	--
26/04	-	10 - home	--
30/04	V [e]	-	
03/05	-	11 [e] - away	E
07/05	VI [f]	-	
10/05	-	18 [f] - home	F

Table 2. List of the training sessions and matches for the collection of the data used in the present study.

Tubes were centrifuged at 1,000× g for 2 min, and the collected saliva samples were subdivided into aliquots and stored at -20 °C. All of the samples were tested at the end of the study, in a single session for each hormone. The salivary cortisol (normal levels, 1.2-14.7 ng/ml), testosterone (normal levels, 47.2-136.2 pg/ml) and DHEA-S (normal levels, 0.2-1.7 ng/ml) were determined by ELISA (commercial kits; D.R.G. International. Inc, Mountainside, NJ, USA). We have taken steroid measurements from saliva samples, which have been shown to provide reliable measures of the circulating levels of free steroids, rather than their total circulating levels, which are confounded by the presence of binding proteins [16]. Moreover, this saliva collection is non-invasive, stress-free and easily repeatable, thus making it the ideal technique for repeat sampling in time-shift studies, in field contexts, as soccer, eliminating the psychological effect of the needle on cortisol production of needle-sensitive people.

Statistical analysis

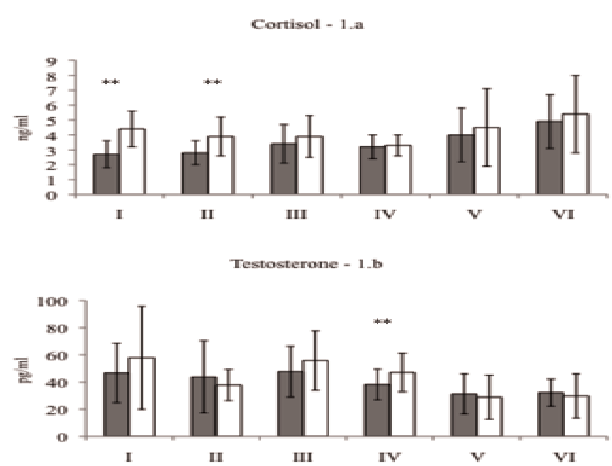
The statistical analysis was performed using SPSS 15 for Windows software (SPSS Inc., Chicago, IL, USA). Student's t-tests for paired data were performed to compare the samples given before and after each single training session and match. Two-way repeated-measures ANOVA was carried out to analyze the time courses of the hormones investigated through the study, and to compare the training sessions with the matches. When statistical significance was detected, one-way repeated-measures ANOVA was carried out to separately verify the effects of training sessions and matches on the hormonal trends. We also compared the time courses of the variation (Δ) of each hormone as a consequence of the training sessions and matches. The variation of each hormone was calculated by subtracting the value of the pre-session sample from that of the post-session sample. To have comparisons of the hormones investigated for the training sessions and matches that are as precise as possible, the statistical analyses were only performed with the data of players who participated in the training sessions and the matches considered; the goalkeepers were excluded from the analyses.

Statistical significance was set at $p \leq 0.05$.

Results

Training sessions

In the comparison of the data before and after the training sessions, significant increases in the cortisol levels were seen for two of the six training sessions, while for the remaining training sessions, there were non-significant increases for cortisol (Figure 1a). Significant variations of the testosterone (i.e. increase) and of DHEA-S (i.e. decrease) were registered only in one of the training sessions (Figures 1b, 1c). No significant differences have been detected in any of the training sessions for cortisol-to-testosterone ratio (Figure 1d).



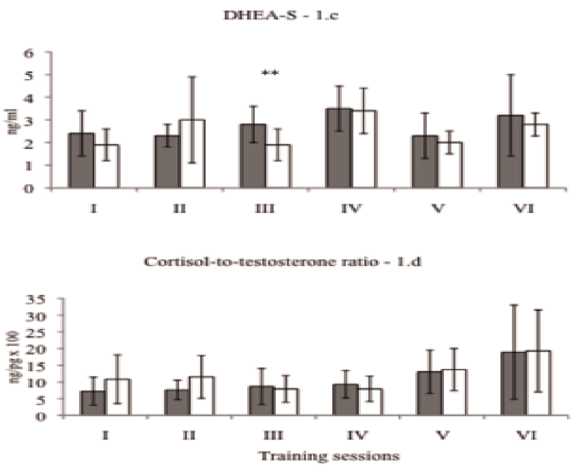


Figure 1. Hormonal variation from pre to post-training sessions (I-VI). Cortisol (a), testosterone (b), DHEA-S (c), and cortisol-to-testosterone ratios (d). Gray columns, pre-training; white columns, post-training. Data are means ±s.d., ** p <0.05.

Matches

When comparing the data before and after the matches, significant increases in the cortisol levels were seen for all but one match (Table 3). The testosterone levels significantly decreased in eight of the 12 matches, with the remaining four matches showing non-significant variation (Table 3). Of note, three of the four matches where the testosterone levels were not significantly modified were matches played away from home (Tables 2, 3). The DHEA-S levels showed no significant changes across 11 of the matches, with the remaining match showing a small, but significant decrease (Table 3). When comparing the cortisol-to-testosterone ratios before and after the matches, it significantly increased for 10 of the 12 matches, with non-significant increases in the two remaining matches (Table 3).

Comparisons between training and matches

As the data above provided indications of differences between the training sessions and the matches, comparative analysis of the data obtained in close temporal sequence was performed. Only those training sessions and matches that occurred at a maximum of three days apart were considered. Thus, through the study, and hence through the playing season, six training session/match paired events were available for analysis (Table 2).

For cortisol levels, the multivariate analysis showed significant effects of time and time × groups (i.e. training session or match) on both the resting levels (time effect $F(5, 120) = 13.117$, $p < 0.001$; time × groups effect $F(5, 120) = 12.269$, $p < 0.001$) and Δ (time effect $F(5, 120) = 2.700$, $p = 0.02$; time × groups effect $F(5, 120) = 5.284$; $p < 0.001$). Overall, the pre-match cortisol levels were significantly greater than those of pre-training ($p = 0.02$). While the pre-training cortisol levels showed a steady increase through the season ($p < 0.001$), the pre-match cortisol levels showed significant variations without any specific trend ($p < 0.001$) (Figure 2a). Over this same period, the training sessions did not result in significant Δ for the cortisol levels ($p = 0.07$), while overall the match sessions resulted in increased Δ cortisol ($p < 0.001$), although the trend of this cortisol response was not homogeneous (Figure 2b).

For the testosterone levels, the multivariate analysis showed significant effects of time and time × groups on both the resting levels (time effect $F(5, 120) =6.663$, $p < 0.001$; time × groups effect $F(5, 120) = 3.922$, $p = 0.003$) and Δ (time effect $F(5, 120) = 2.356$, $p = 0.04$; time × groups effect $F(5,120) = 3.365$; $p =0.007$). While the pre-training testosterone levels decreased significantly through the season ($p = 0.04$), the pre-match levels showed significant variations without any specific trend ($p < 0.001$) (Figure 3a).

Match	Cortisol (ng/ml)			Testosterone (pg/ml)			DHEA-S (ng/ml)			Cortisol/Testosterone (×100)		
	pre	*post*	*p*	*pre*	*post*	*p*	*pre*	*post*	*p*	*pre*	*post*	*p*
1	5.9 ±1.8	14.2 ±4.0	**<0.001**	58.4 ±34.5	65.2 ±31.2	n.s.	3.1 ±0.9	3.0 ±0.7	n.s.	15.6 ±18.0	31.8 ±27.9	**0.03**
2	2.8 ±1.6	6.2 ±2.7	**0.001**	51.5 ±16.0	23.4 ±6.8	**<0.001**	2.0 ±1.7	2.3 ±1.6	n.s.	6.3 ±4.0	27.9 ±18.9	**<0.001**
3	3.1 ±0.7	5.1 ±2.5	**0.02**	35.1 ±6.8	15.8 ±6.5	**<0.001**	2.0 ±0.6	2.1 ±1.4	n.s.	9.3 ±3.0	41.8 ±34.3	**0.01**
4	5.5 ±1.5	6.7 ±2.4	**0.02**	63.7 ±24.7	63.3 ±21.3	n.s.	2.7 ±0.7	2.7 ±.8	n.s.	9.8 ±4.1	18.0 ±5.7	n.s.
5	8.4 ±2.3	10.6 ±7.0	n.s.	37.5 ±27.5	21.1 ±15.9	**0.01**	1.9 ±0.8	1.6 ±0.6	n.s.	31.2 ±22.4	61.1 ±47.0	n.s.
6	7.0 ±2.8	18.9 ±6.2	**0.01**	29.0 ±19.2	14.6 ±6.7	**0.02**	1.6 ±0.7	1.9 ±0.6	n.s.	30.2 ±18.0	97.6 ±62.8	**0.003**
7	4.0 ±1.6	7.4 ±2.	**0.001**	52.7 ±16.0	24.6 ±6.8	**<0.001**	3.1 ±1.7	3.5 ±1.6	n.s.	8.5 ±4.3	31.4 ±18.5	**<0.001**
8	4.4 ±2.6	9.1 ±4.3	**0.003**	18.8 ±9.3	11.2 ±3.8	**0.01**	2.0 ±0.8	1.2 ±0.5	**0.01**	27.0 ±14.5	101.6 ±86.6	**0.006**
9	5.2 ±2.0	13.1 ±3.8	**<0.001**	53.9 ±34.7	59.3 ±33.8	n.s.	2.3 ±1.1	2.0 ±0.8	n.s.	16.1 ±13.1	38.1 ±35.9	**0.01**
10	5.2 ±1.4	7.3 ±3.4	**0.04**	40.9 ±27.6	21.6 ±18.1	**0.005**	2.0 ±0.7	1.5 ±0.6	n.s.	20.6 ±13.4	44.5 ±30.4	**0.004**
11	6.4 ±1.8	14.0 ±4.3	**<0.001**	52.7 ±29.3	58.3 ±28.	n.s.	2.2 ±0.8	2.3 ±0.6	n.s.	17.9 ±13.9	33.2 ±29.4	**0.03**
18	2.2 ±1.7	5.3 ±2.6	**0.001**	49.0 ±16.1	25.1 ±11.1	**0.003**	1.3 ±1.4	1.4 ±1.4	n.s.	5.4 ±5.0	23.4 ±13.3	**0.001**

Table 3. Hormones variation across the 18 matches evaluated. Data are mean ±s.d.

At the same time, the training sessions saw a different Δ in the testosterone levels than the matches: with the matches, the testosterone levels were generally decreased ($p < 0.001$), and in the training sessions there were alternating increases and decreases. Through the season, the matches also showed reductions in these decreases in testosterone levels, except in the match of pair F (Figure 3b).

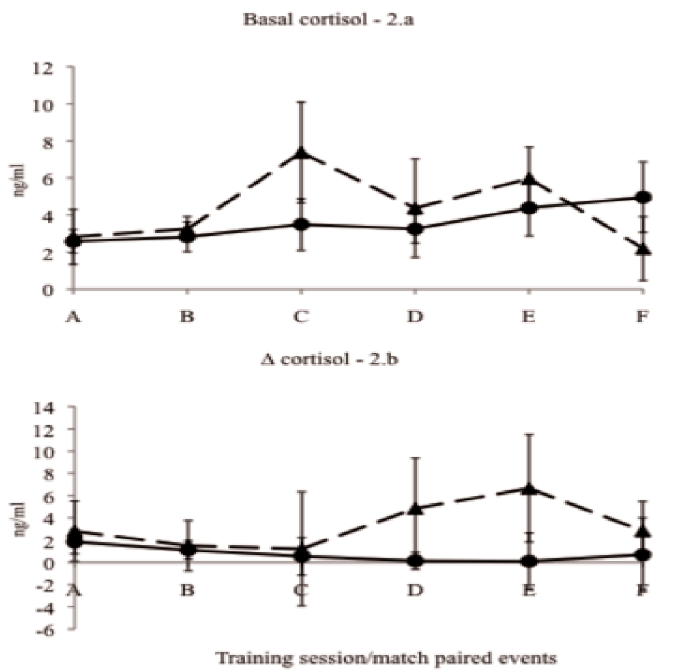


Figure 2. Salivary cortisol.
Seasonal trend of resting cortisol and its variation (Δ) according to the matched pairs of training sessions (i.e. solid lines) and matches (i.e. dashed lines). (a) The pre-match levels of cortisol were greater than those of pre-training ($p = 0.02$), while pre-training levels showed a steady increase through the season ($p < 0.001$). (b) The Δ of cortisol for the training sessions showed lower variations than for the matches ($p = 0.001$), while through the season the training sessions showed reduced increases of cortisol ($p = 0.02$).

Similarly, for the DHEA-S levels, the multivariate analysis showed significant effects of time and time × groups on the resting levels (time effect $F(5, 120) = 2.483$, $p = 0.03$; time × groups effect $F(5, 120) = 6.580$, $p < 0.001$). The pre-training DHEA-S levels were significantly greater than those of the pre-matches ($p = 0.002$), and while the pre-training DHEA-S levels showed an irregular increase through the season ($p < 0.001$), those of the pre-matches showed an irregular decrease ($p < 0.001$) (Figure 4). When the same statistical analysis was performed on the Δ of the DHEA-S through the season, no significance changes were seen.

The multivariate analysis on the cortisol-to-testosterone ratios showed significant effects of time and time × groups on the resting levels (time effect $F(5, 120) = 6.421$, $p < 0.001$; time × groups effect $F(5, 120) = 6.803$, $p < 0.001$). The pre-matches ratios were significantly greater than those of pre-training ($p = 0.03$), although they both then showed increases through the season; however, for the last match, the pre-match values of the cortisol-to-testosterone ratio inverted this trend (Figure 5) ($p < 0.001$). No statistical differences were seen for the RM-ANOVA for the Δ of cortisol-to-testosterone ratios for training sessions and matches.

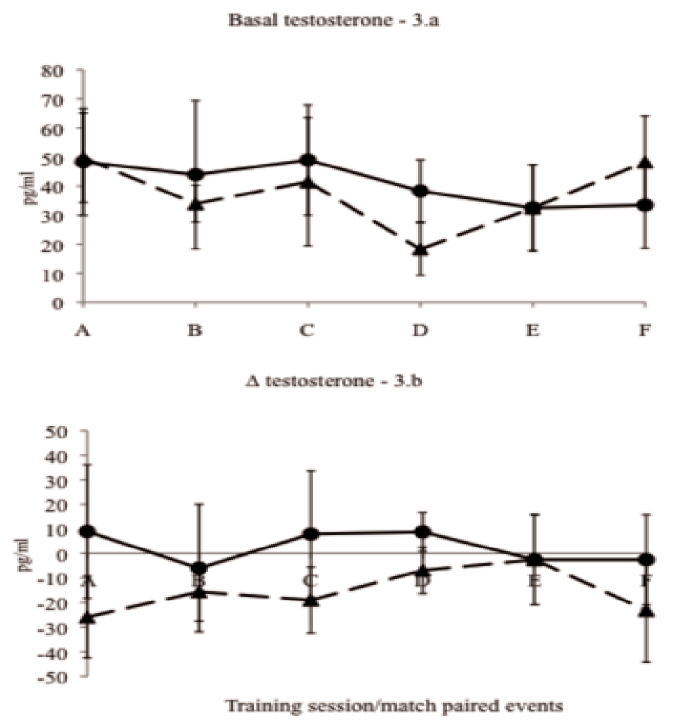


Figure 3. Salivary testosterone.
Seasonal trend of resting testosterone and its variation (Δ) according to the matched pairs of training sessions (i.e. solid lines) and matches (i.e. dashed lines). (a) Both the pre-training and pre-match levels of testosterone decreased through the season ($p = 0.003$). (b) The Δ of testosterone showed significant differences, both within ($p = 0.007$) and between ($p < 0.001$) groups. The training sessions showed different variation of Δ testosterone than the matches, with a general increase compared to a decrease, respectively: for the matches, the decreases in testosterone became less pronounced.

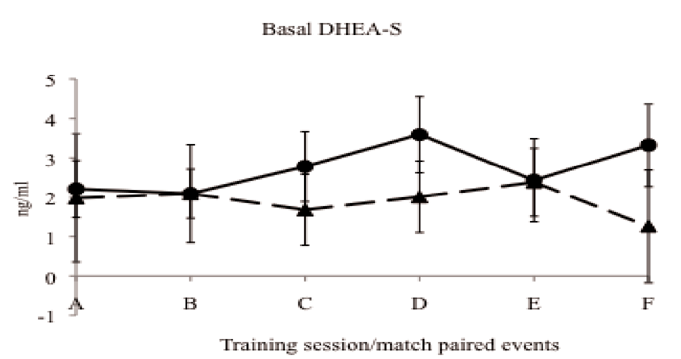


Figure 4. Salivary DHEA-S.
Seasonal trend of resting DHEA-S according to the matched pairs of training sessions (i.e. solid lines) and matches (i.e. dashed lines). The

pre-training DHEA-S levels were greater than those of pre-matches (p =0.002), and while the pre-training levels showed an irregular increase through the season, those of the pre-matches showed an irregular decrease (p <0.001).

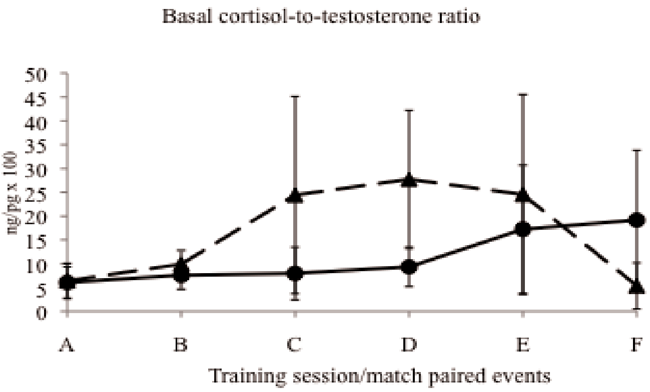


Figure 5. Salivary cortisol-to-testosterone ratios.
Seasonal trend of resting cortisol-to-testosterone ratios according to the matched pairs of training sessions (i.e. solid lines) and matches (i.e. dashed lines). The pre-match cortisol-to-testosterone ratios were greater than those of pre-training (p =0.03), while both increased through the season (p <0.001).

Discussion

The hypothalamic–pituitary–adrenal axis and hypothalamic–pituitary–testis axis have been extensively studied regarding their modifications after physical and psychic stress, and combinations of both. However, even with consideration of the same sport (e.g. soccer), cortisol and testosterone levels have been described as differently modified, depending on the scenario and the evaluation conditions.

We show here that the cortisol levels significantly increase across the training sessions for the few sessions at the beginning of the season. In the meantime, the resting cortisol levels significantly and progressively increase from the beginning to the end of the season. Haneishi et al. [11] did not show any significant changes in cortisol levels after a training session, and Moreira et al. [13] reported similar data, in a group of male top-level players, after a training match. As a contrast to these studies, Inspiridis et al. [12] showed a transient increase in cortisol levels in male top-levels players after an experimental game. Our data are therefore somewhere in-between these previous studies, although this is not a constant feature. Here, the period of the season investigated appears to have a significant role, as discussed further below.

The testosterone levels increased significantly after only one of the training sessions, while its basal level progressively decreased throughout the study. Consequential to the previously discussed data, for the training sessions, we also showed a significant increase in the basal cortisol-to-testosterone ratio. This trend is consistent with a previous report [15] that showed a reduction in the testosterone-to-cortisol ratio towards the end of a competitive season.

Different hormonal trends were seen for the matches. An increase in cortisol levels after a match has been reported in several studies [10-12,17] and has been shown for both male and female players, although, in female players, no changes have also been described [11]. The trend of testosterone after a competitive match has remained subject to more debate. Thus, while some studies have reported an increase in testosterone levels after a soccer game [10], others have described no significant changes in female players [18], and others still have reported different trends according to the match losers and winners; the losers showed a decrease in testosterone levels, while the winners showed significantly increased testosterone levels [14].

We also carried out a more significant and precise comparison of these hormonal changes after training sessions and matches by comparing the training sessions and matches that occurred only three days apart. This analysis allowed us to highlight some significant differences for all of the hormones investigated. However, it is relatively difficult to interpret all of these data from a single point of view, although data obtained before and after the training sessions are more consistent than those obtained in relation to the matches. Moreover, our unpublished data reinforce the hypothesis of the presence of several independent factors influencing the hormone levels before and after the matches; indeed, they showed the influence of also the tactical performance of the player, the outcome of the game and its trend through the season (i.e. a successful or unsuccessful team). Bearing these limitations in mind, two specific points can be outlined and discussed here.

a. The time of the season is a determinant in the evaluation of these data: different basal levels were seen through the season for cortisol, testosterone, DHEA-S and cortisol-to-testosterone ratio across both the training sessions and the matches, although the training sessions showed a more regular trend. Thus, a possible explanation for the conflicting data reported by several studies can be found by bearing in mind that a progressive increase in the cortisol-to-testosterone ratio and decrease in testosterone levels have been described in soccer players after a competition half season [15], and in rugby players as a consequence of intensive training [19]. Moreover, cortisol-to-testosterone ratios might be useful for monitoring adaptation and recovery to training and match-play stress in players of team sports [20,21]. The progressive increase in the basal cortisol levels and decrease in the basal testosterone levels, and consequently the increase in their ratio, can be interpreted according to Coutts et al. [19], as a measure that is indicative of players who are progressively worsening in their ability to tolerate the training loads.

b. The hormonal modifications after training sessions and matches might differ completely. Our data are therefore in agreement with the results reported by Haneishi et al. [11], who described a larger increase in cortisol after matches than after training, and with those described by Moreira et al. [13], who showed a non-significant increase in cortisol levels after a training match. The larger increases in cortisol levels after matches might also explain the different trend of testosterone; indeed, after high-intensity exercise, a decrease in testosterone levels contemporary to an increase in cortisol levels has been described [22,23]. This was explained through the possible involvement of the hypothalamus–pituitary–testis axis [23]. It was thus proposed that lowered testosterone levels and elevated cortisol levels will help energy stores to be replenished and will supply the precursors for later use in gluconeogenesis and protein synthesis. These data are also supported by a study of Kyröläinen et al. [24], who showed an increase in cortisol levels and a decrease in testosterone and luteinizing hormone levels during military field exercises associated with high-energy expenditure. Fitting into this context are also the different results obtained by Edwards et al. [10], who showed an increase in testosterone levels in female players after a 5-0 victory (i.e. non-strenuous effort), but a decrease after a 1-2 loss (i.e. strenuous effort). In both of these cases, and similar to our data, the cortisol levels significantly increased. Male players were evaluated by Edwards et al. [10] as well: their cortisol levels significantly increased, while their testosterone levels showed a non-significant increase, which was after a single home game that ended with a double-overtime 1-0 victory. It also has to be noted that the game evaluated by Edwards et al. [10] was played at the end of the season. In comparison, although we observed a significant reduction in testosterone levels across most of our matches (i.e. in 8/12), two of the three matches where the testosterone levels showed non-significant increases occurred in the last four matches of the season. At the same time, we also observed reduced changes in the hormone levels for both cortisol's, mainly for the training sessions, and testosterone, mainly for the matches, with time. Thus, if the decrease in the testosterone levels is functional for the replenishment of energy stores, as previously discussed, we can argue that this phenomenon is blunted towards the end of the season, when the adaptation ability decreases.

In conclusion, in this study we have monitored the changes in cortisol, testosterone and DHEA-S levels, and thus adrenal and testis function, in male soccer players through an entire playing season, with evaluation across both their training sessions and their matches. We have highlighted and discussed the response of the players to the different stress stimuli, also considering the conflicting data published so far. We believe to have shown that further evaluation of adrenal and testis functions will have to take into account the time of the season, thus providing an explanation for most of the differences that have been reported in previous studies.

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